

WHAT IS CLAIMED IS

1 1. A composition comprising a MTB39 antigen (SEQ ID NO:12 or
2 14) or an immunogenic fragment thereof from a *Mycobacterium* species of the
3 tuberculosis complex, and a MTB32A antigen (SEQ ID NO:2 or 4) or an immunogenic
4 fragment thereof from a *Mycobacterium* species of the tuberculosis complex.

1 2. The composition of claim 1, comprising a MTB39 antigen (SEQ
2 ID NO:12 or 14) or an immunogenic fragment thereof from a *Mycobacterium* species of
3 the tuberculosis complex, and a polypeptide comprising at least 195 amino acids from the
4 N-terminus of a MTB32A antigen (SEQ ID NO:2 or 4) from a *Mycobacterium* species of
5 the tuberculosis complex.

1 3. The composition of claim 2, further comprising a polypeptide
2 comprising at least about 132 amino acids from the C-terminus of MTB32A antigen
3 (SEQ ID NO:2 or 4) from a *Mycobacterium* species of the tuberculosis complex.

1 4. The composition of claims 1, 2, or 3, wherein the antigens are
2 covalently linked, thereby forming a fusion polypeptide.

1 5. The composition of claim 4, wherein the fusion polypeptide has the
2 amino acid sequence of MTB59F (SEQ ID NO:20).

1 6. The composition of claim 4, wherein the fusion polypeptide has the
2 amino acid sequence of MTB72F (SEQ ID NO:16).

1 7. The composition of claim 4, wherein the fusion polypeptide has the
2 amino acid sequence of MTB72FMutSA (SEQ ID NO:18).

1 8. The composition of claim 6 or 7, further comprising BCG.

1 9. The composition of claim 6 or 7, further comprising at least one
2 additional antigen from a *Mycobacterium* species of the tuberculosis complex, wherein
3 the antigen is selected from the group consisting of MTB8.4 antigen (SEQ ID NO:22),
4 MTB9.8 antigen (SEQ ID NO:24), MTB9.9 antigen (SEQ ID NO:27), MTB40 antigen
5 (SEQ ID NO:29), MTB41 antigen (SEQ ID NO:31), 38-1 (SEQ ID NO:35), TbRa3 (SEQ
6 ID NO:37), 38 kD (SEQ ID NO:39), DPEP (SEQ ID NO:41), TbH4 (SEQ ID NO:43),

- 7 DPPD(SEQ ID NO:45), MTB82, Erd14, ESAT-6 antigen (SEQ ID NO:33), MTB85
8 complex antigen, or α -crystalline antigen, or an immunogenic fragment thereof.
- 1 10. The composition of claim 6 or 7, further comprising an adjuvant.
- 1 11. The composition of claim 4, wherein the antigens are covalently
2 linked via a chemical linker.
- 1 12. The composition of claim 11, wherein the chemical linker is an
2 amino acid linker.
- 1 13. The composition of claim 1, further comprising at least one
2 additional antigen from a *Mycobacterium* species of the tuberculosis complex, wherein
3 the antigen is selected from the group consisting of MTB8.4 antigen (SEQ ID NO:22),
4 MTB9.8 antigen (SEQ ID NO:24), MTB9.9 antigen (SEQ ID NO:27), MTB40 antigen
5 (SEQ ID NO:29), MTB41 antigen (SEQ ID NO:31), 38-1 (SEQ ID NO:35), TbRa3 (SEQ
6 ID NO:37), 38 kD (SEQ ID NO:39), DPEP (SEQ ID NO:41), TbH4 (SEQ ID NO:43),
7 DPPD(SEQ ID NO:45), MTB82, Erd14, ESAT-6 antigen (SEQ ID NO:33), MTB85
8 complex antigen, or α -crystalline antigen, or an immunogenic fragment thereof.
- 1 14. The composition of claim 1, further comprising an adjuvant.
- 1 15. The composition of claim 14, wherein the adjuvant comprises
2 QS21 and MPL.
- 1 16. The composition of claim 14, wherein the adjuvant is selected from
2 the group consisting of AS2, ENHANZYN, MPL, 3D-MPL, IFA, QS21, CWS, TDM,
3 AGP, CPG, Leif, saponin, and saponin mimetics.
- 1 17. The composition of claim 1, further comprising BCG or pVac.
- 1 18. The composition of claim 1, further comprising an NS1 antigen or
2 an immunogenic fragment thereof.
- 1 19. The composition of claim 1, wherein the *Mycobacterium* species is
2 *Mycobacterium tuberculosis*.

21. The expression cassette of claim 20, comprising a nucleic acid encoding a MTB39 antigen (SEQ ID NO:12 or 14) or an immunogenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex, and a nucleic acid encoding a polypeptide comprising at least 195 amino acids from the N-terminus of a MTB32A antigen (SEQ ID NO: 2 or 4) from a *Mycobacterium* species of the tuberculosis complex.

1 22. The expression cassette of claim 21, further comprising a nucleic
2 acid encoding a polypeptide comprising at least 132 amino acids of the C-terminus of a
3 MTB32A antigen (SEQ ID NO:2 or 4) from a *Mycobacterium* species of the tuberculosis
4 complex.

1 23. The expression cassette of claim 20, wherein the nucleic acid
2 encodes a fusion polypeptide comprising a MTB39 antigen (SEQ ID NO:12 or 14) or an
3 immunogenic fragment thereof and a nucleic acid encoding a MTB32A antigen (SEQ ID
4 NO:2 or 4) or an immunogenic fragment thereof.

24. The expression cassette of claim 23, wherein the nucleic acid encodes a fusion polypeptide comprising a MTB39 antigen (SEQ ID NO:12 or 14) or an immunogenic fragment thereof, and a polypeptide comprising at least 195 amino acids from the N-terminus of a MTB32A antigen (SEQ ID NO:2 or 4).

1 25. The expression cassette of claim 24, wherein the fusion
2 polypeptide further comprises a polypeptide comprising at least 132 amino acids of the C-
3 terminus of a MTB32A antigen (SEQ ID NO:2 or 4).

1 26. The expression cassette of claim 24, wherein the nucleic acid
2 encodes a fusion polypeptide having the amino acid sequence of MTB59F (SEQ ID
3 NO:20).

1 27. The expression cassette of claim 26, wherein the nucleic acid has
2 the sequence of the nucleic acid encoding MTB59F (SEQ ID NO:19).

1 28. The expression cassette of claim 25, wherein the nucleic acid
2 encodes a fusion polypeptide having the amino acid sequence of MTB72F (SEQ ID
3 NO:16).

1 29. The expression cassette of claim 28, wherein the nucleic acid has
2 the sequence of the nucleic acid encoding MTB72F (SEQ ID NO:15).

1 30. The expression cassette of claim 28, wherein the nucleic acid has
2 the sequence of the nucleic acid encoding MTB72F MutSA (SEQ ID NO:18).

1 31. The expression cassette of claim 29 or 30, further comprising a
2 nucleic acid encoding at least one additional antigen from a *Mycobacterium* species of the
3 tuberculosis complex, wherein the antigen is selected from the group consisting
4 of MTB8.4 antigen (SEQ ID NO:22), MTB9.8 antigen (SEQ ID NO:24), MTB9.9 antigen
5 (SEQ ID NO:27), MTB40 antigen (SEQ ID NO:29), MTB41 antigen (SEQ ID NO:31),
6 38-kDa (SEQ ID NO:35), TbRa3 (SEQ ID NO:37), 38 kDa (SEQ ID NO:39), DPEP (SEQ ID
7 NO:41), TbH4 (SEQ ID NO:43), DPPD (SEQ ID NO:45), MTB82, Erd14, ESAT-6
8 antigen (SEQ ID NO:33), MTB85 complex antigen, or α -crystalline antigen, or an
9 immunogenic fragment thereof.

1 32. The expression cassette of claim 20, further comprising a nucleic
2 acid encoding at least one additional antigen from a *Mycobacterium* species of the
3 tuberculosis complex, wherein the antigen is selected from the group consisting
4 of MTB8.4 antigen (SEQ ID NO:22), MTB9.8 antigen (SEQ ID NO:24), MTB9.9 antigen
5 (SEQ ID NO:27), MTB40 antigen (SEQ ID NO:29), MTB41 antigen (SEQ ID NO:31),
6 38-kDa (SEQ ID NO:35), TbRa3 (SEQ ID NO:37), 38 kDa (SEQ ID NO:39), DPEP (SEQ ID
7 NO:41), TbH4 (SEQ ID NO:43), DPPD (SEQ ID NO:45), MTB82, Erd14, ESAT-6
8 antigen (SEQ ID NO:33), MTB85 complex antigen, or α -crystalline antigen, or an
9 immunogenic fragment thereof.

1 33. The expression cassette of claim 20, further comprising a nucleic
2 acid encoding an NS1 antigen.

1 34. The expression cassette of claim 20, wherein the *Mycobacterium*
2 species is *Mycobacterium tuberculosis*.

1 35. A method for eliciting an immune response in a mammal, the
2 method comprising the step of administering to the mammal an immunologically
3 effective amount of a pharmaceutical composition comprising a MTB39 antigen (SEQ ID
4 NO:12 or 14) or an immunogenic fragment thereof from a *Mycobacterium* species of the
5 tuberculosis complex, and a MTB32A antigen (SEQ ID NO:2 or 4) or an immunogenic
6 fragment thereof from a *Mycobacterium* species of the tuberculosis complex.

1 36. The method of claim 35, wherein the mammal has been immunized
2 with BCG.

1 37. The method of claim 35, wherein the mammal is a human.

1 38. The method of claim 35, wherein the composition is administered
2 prophylactically.

1 39. The method of claim 35, comprising a MTB39 antigen (SEQ ID
2 NO:12 or 14) or an immunogenic fragment thereof from a *Mycobacterium* species of the
3 tuberculosis complex, and a polypeptide comprising at least 195 amino acids from the N-
4 terminus of a MTB32A antigen (SEQ ID NO:2 or 4) from a *Mycobacterium* species of
5 the tuberculosis complex.

1 40. The method of claim 39, further comprising a polypeptide
2 comprising at least about 132 amino acids from the C-terminus of MTB32A antigen
3 (SEQ ID NO: 2 or 4) from a *Mycobacterium* species of the tuberculosis complex.

1 41. The method of claim 35 or 39, wherein the antigens are covalently
2 linked, thereby forming a fusion protein.

1 42. The method of claim 41, wherein the fusion polypeptide has the
2 amino acid sequence of MTB59F (SEQ ID NO:20).

1 43. The method of claim 40, wherein the antigens are covalently
2 linked, thereby forming a fusion protein.

- 1 44. The method of claim 43, wherein the fusion polypeptide has the
2 amino acid sequence of MTB72F (SEQ ID NO:16).
- 1 45. The method of claim 43, wherein the fusion polypeptide has the
2 amino acid sequence of MTB72FMutSA (SEQ ID NO:18).
- 1 46. The method of claim 35, wherein the pharmaceutical composition
2 further comprises an adjuvant.
- 1 47. The method of claim 46, wherein the adjuvant comprises QS21 and
2 MPL.
- 1 48. The method of claim 46, wherein the adjuvant is selected from the
2 group consisting of AS2, ENHANZYN, MPL, 3D-MPL, IFA, QS21, CWS, TDM, AGP,
3 CPG, Leif, saponin, and saponin mimetics.
- 1 49. A method for eliciting an immune response in a mammal, the
2 method comprising the step of administering to the mammal an immunologically
3 effective amount of an expression cassette comprising a nucleic acid encoding a MTB39
4 antigen (SEQ ID NO:12 or 14) or an immunogenic fragment thereof from a
5 *Mycobacterium* species of the tuberculosis complex, and a nucleic acid encoding a
6 MTB32A antigen (SEQ ID NO:2 or 4) or an immunogenic fragment thereof from a
7 *Mycobacterium* species of the tuberculosis complex.
- 1 50. The method of claim 49, wherein the mammal has been immunized
2 with BCG.
- 1 51. The method of claim 49, wherein the mammal is a human.
- 1 52. The method of claim 49, wherein the composition is administered
2 prophylactically.
- 1 53. The method of claim 49, wherein the nucleic acid encodes a fusion
2 polypeptide comprising a MTB39 antigen (SEQ ID NO:12 or 14) or an immunogenic
3 fragment thereof, and a polypeptide comprising at least 195 amino acids from the N-
4 terminus of a MTB32A antigen (SEQ ID NO:2 or 4) .

1 54. The method of claim 53, further comprising a nucleic acid
2 encoding a polypeptide comprising at least 132 amino acids of the C-terminus of a
3 MTB32A antigen (SEQ ID NO:2 or 4) from a *Mycobacterium* species of the tuberculosis
4 complex.

1 55. The method of claim 49, wherein the nucleic acid encodes a fusion
2 polypeptide comprising a MTB39 antigen (SEQ ID NO: 12 or 14) or an immunogenic
3 fragment thereof and a nucleic acid encoding a MTB32A antigen (SEQ ID NO:2 or 4) or
4 an immunogenic fragment thereof.

1 56. The method of claim 55, wherein the nucleic acid encodes a fusion
2 polypeptide comprising a MTB39 antigen (SEQ ID NO:12 or 14) or an immunogenic
3 fragment thereof, and a polypeptide comprising at least 195 amino acids from the N-
4 terminus of a MTB32A antigen (SEQ ID NO: 2 or 4).

1 57. The method of claim 56, wherein the fusion polypeptide further
2 comprises a polypeptide comprising at least 132 amino acids of the C-terminus of a
3 MTB32A antigen (SEQ ID NO:2 or 4).

1 58. The method of claim 56, wherein the nucleic acid encodes a fusion
2 polypeptide having the amino acid sequence of MTB59F (SEQ ID NO:20).

1 59. The method of claim 58, wherein the nucleic acid has the
2 nucleotide sequence of the nucleic acid encoding MTB59F (SEQ IDNO:19).

1 60. The method of claim 57, wherein the nucleic acid encodes a fusion
2 polypeptide having the amino acid sequence of MTB72F (SEQ ID NO:16) .

1 61. The method of claim 57, wherein the nucleic acid encodes a fusion
2 polypeptide having the amino acid sequence of MTB72FMutSA (SEQ ID NO:18).

1 62. The method of claim 60, wherein the nucleic acid has the
2 nucleotide sequence of the nucleic acid encoding MTB72F (SEQ IDNO:15).

1 63. The method of claim 60, wherein the nucleic acid has the
2 nucleotide sequence of the nucleic acid encoding MTB72FMutSA (SEQ ID NO:17).

64. An isolated nucleic acid encoding a MTB32A antigen from a *Mycobacterium* species of the tuberculosis complex, wherein at least one amino acid in the active site triad of the MTB32A antigen (SEQ ID NO:2 or 4) has been substituted by a different amino acid.

1 65. The nucleic acid of claim 64, wherein an serine residue
2 corresponding to amino acid position 183 of SEQ ID NO:4 or position 207 of SEQ ID
3 NO:2 has been substituted by another amino acid.

1 66. The nucleic acid of claim 65, wherein an alanine residue has been
2 substituted for the serine residue.

1 67. The nucleic acid of claim 66, wherein the nucleic acid comprises a
2 nucleotide sequence of SEQ ID NO:5.

1 68. A composition comprising the nucleic acid of claim 64.

1 69. A nucleic acid encoding a fusion polypeptide comprising the
2 nucleic acid of claim 64.

70. An isolated MTB32A polypeptide from a *Mycobacterium* species of the tuberculosis complex, wherein at least one amino acid in the active site triad of the MTB32A antigen (SEQ ID NO:2 or 4) has been substituted by a different amino acid.

1 71. The polypeptide of claim 70, wherein a serine residue
2 corresponding to amino acid position 183 of SEQ ID NO:4 or amino acid position 207 of
3 SEQ ID NO:2 has been substituted by another amino acid.

1 72. The polypeptide of claim 71, wherein an alanine residue has been
2 substituted for the serine residue.

1 73. A polypeptide of claim 72, wherein the polypeptide comprises an
2 amino acid sequence of SEQ ID NO:6.

1 74. A composition comprising the polypeptide of claim 70.

1 75. A fusion polypeptide comprising the polypeptide of claim 70.

1 76. An isolated nucleic acid encoding a fusion polypeptide comprising
2 a MTB39 antigen (SEQ ID NO:12 or 14) from a *Mycobacterium* species of the
3 tuberculosis complex, and an antigen comprising at least 195 amino acids from the N-
4 terminus of a MTB32A antigen (SEQ ID NO:2 or 4) from a *Mycobacterium* species of
5 the tuberculosis complex, wherein an amino acid of the active site triad of the MTB32A
6 antigen (SEQ ID NO:2 or 4) has been substituted by a different amino acid.

1 77. The nucleic acid of claim 76, wherein a serine residue
2 corresponding to amino acid at position 183 of SEQ ID NO:4 or position 207 or SEQ ID
3 NO:2 has been substituted by another amino acid.

1 78. The nucleic acid of claim 77, wherein an alanine residue has been
2 substituted for the serine residue.

1 79. A composition comprising the nucleic acid of claim 76.

1 80. A nucleic acid encoding a fusion polypeptide comprising the
2 nucleic acid of claim 76.

1 81. A nucleic acid encoding a fusion polypeptide, wherein the nucleic
2 acid comprises a nucleotide sequence of SEQ ID NO:17.

1 82. A nucleic acid encoding a fusion polypeptide comprising an amino
2 acid sequence of SEQ ID NO:18.

1 83. An isolated polypeptide encoding a fusion polypeptide comprising
2 a MTB39 (SEQ ID NO: 12 or 14) antigen from a *Mycobacterium* species of the
3 tuberculosis complex, and an antigen comprising at least 195 amino acids from the N-
4 terminus of a MTB32A antigen (SEQ ID NO:2 or 4) from a *Mycobacterium* species of
5 the tuberculosis complex, wherein an amino acid of the active site triad of the MTB32A
6 antigen (SEQ ID NO:2 or 4) has been substituted by a different amino acid.

1 84. The polypeptide of claim 83, wherein an serine residue
2 corresponding to amino acid position 183 of SEQ ID NO:4 or amino acid position 207 of
3 SEQ ID NO:2 has been substituted by another amino acid.

1 85. The polypeptide of claim 83, wherein an alanine residue has been
2 substituted for the serine residue.

1 86. A composition comprising the polypeptide of claim 83.

1 87. A fusion polypeptide comprising the polypeptide of claim 83.

1 88. A fusion polypeptide comprising an amino acid sequence of SEQ
2 ID NO:18.

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